Journal of the Imperial Fisheries Institute, Vol. XXIII, No. 4.
February, 1928.

STUDIES ON FISH-DISEASE.

I. "IKARI-MUSHI" (*Lernaea elegans* Leigh-Sharpe)., a new parasitic Copepod of Japanese Eel.

(Contributions from the Toyohashi Piscicultural Experiment Station No. 1.)

By

Yoshiichi Matsui and Asao Kumada.

About the middle of July 1922 we were requested by a fish culturist near Toyohashi to analyze the water of his pond to ascertain whether it is fit to rear fish or not, on the ground that the eels stopped feeding suddenly, but we were unable to find any positive reactions as far as the quality of the water is concerned.

A few days later, we recieved the sample of diseased eels which were found in the pond. Apparently it seemed like suffering from a certain disease in buccal cavity known as "Kuchikusare", after a closer examination of the buccal cavity we discovered the presence of innumerable greenish parasites in it.

Since then the parasite has increased and has spread very rapidly, attacking neighboring ponds and by the fall of 1926, it was reported to be found very commonly in such places as Tokyo and Prefectures Kanagawa, Shizuoka, Aichi, Mie, Shiga, Hyogo and Ibaraki, and its harmful effect was considered very seriously.

This parasite resembles very closely to *Lernaea cyprinacea L.*, but since we found that it does not agree with the species above, we sent the sample of the parasite to Dr. W. H. Leigh-Sharpe in England who examined the worm as a new species, and named it as *Lernaea elegans.**

As this parasite caused the disease which has some serious effects upon the nutrition of cultured eels, it will not be worthless to report the results of our investigation giving to it a new Japanese name, "Ikari-mushi" (Anchor-worm), from its resemblance to an anchor. On this occasion we greatly appreciate the kindness shown to us by

* *Lernaea* (*Lerneocera*) *elegans* n. sp. a parasitic Copepod of Anguilla japonica: Parasitology. Vol. 17, pp. 245–251, 1925.

Dr. Leigh-Sharpe and our friend Mr. Seizo Takeda. Our thanks are also due to Prof. Chiyomatsu Ishikawa for his kindness in looking over our manuscript.

This parasite is found in the buccal cavity and rarely on the skin of the eel (*Anguilla japonica*) and also lives as an ectoparasite among the scales of *Cyprinus carpio* L., *Carassius auratus* (L.), *Pseudorasbora anguillicaudata* (Canter), *Plecoglossus altivelis* T & S., *Oryzias latipes* T. & S. and *Pseudorasbora parva* (T. & S.).

Eels that are infected by this parasite show bloodish marks on the ventral part of the lower jaw, both upper and lower jaws shrink to a crooked form, and the head takes particular shape (Pl. V. fig. 1. A and D), the mouth is half open and we can observe externally the parasites clinging in cluster to the buccal cavity.

Analytical observation reveals that the liver is congested, gall-bladder discolored and swollen, digestive organ emptied and blood vessels of the wall of the body cavity conspicuously congested.

Morphology.

1. Body: In adult female it is measured 12 m.m. from the top of head to the end of egg sac. Second and third thoracic-segments slender, fourth and fifth segments plump and take boot-like shape together with pregenital prominence. In third segment, it has torsion of 90 degrees direct or inverse to longitudinal axis.

2. Head: Head is hemispherical and protrudes further than the plane formed by horns. Eyes are situated under skin in the posterior summit of it. First antenna is four-jointed and has numerous plumose setae at anterior margin of each joint, having specially longer ones on terminal joint. Second antenna is three-jointed, having three setae at inner margin of terminal joint, five plumose setae at outer margin and tipped with a claw like spine. Mandible is very small and hard to observe. First maxilla is situated posterior to second maxilla. Maxilliped is slightly long and plump and is composed of distal joint having two mammillated prominences at internal end, terminal joint having movable five claw like spines. Although Dr. Leigh-Sharpe describes "Auricular expansion" and thoracic appendage, we did only observe arch-shaped bandage connecting each basal joint of both maxillipeds. (Fig. 23.)

3. Horns: Ring-shaped conspicuous boarder line is observed between head and horns and its shape is like letter X. According to the age of worms, form of horn varies much, at younger stage it takes the form (Fig. 1.)

4. Swimming legs: In the centre of anterior prominence of horn, first swimming leg is to be observed. Second and third swimming legs attach to ventral side at the

posterior edge of swollen part of body segment. Fifth swimming leg is situated at the posterior end of thoracic segment right in front of vulva. (Fig. 20, 21, 23, 27, 28.)

5. Pregenital prominence: A buttock like prominence exists on ventral side of posterior end of fifth thoracic segment, its degree of development varies according to age.

6. Genital segment: Genital segment of young with body length of 4 mm. is 0.175 mm. long, 0.123 mm. wide, and is largest of all abdominal segments, but in adult it fuses together with first abdominal segment, leaving no boarder line between two. (Fig. 14, 15.)

7. Abdomen: Four segmented in young, but in adult first and third segments fuse together forming three segments with genital and fourth segment. The anal lamina is formed of three parts. First part is swollen having three setae, middle part is cylindrical and last is needle like and plumose. (Figs. 14, 15.)

8. Skin: Skin consists of three layers, outer is thick and chitinous. Middle layer is very thin, while inner layer is formed of a single layer of multinucleated cells and pavement like. This layer is well developed in posterior-part of body, in middle part it exists only in dorsal and abdominal, while none in anterior part. (Figs. 33, 34, 39.) Skin has numerous funnel shaped pore canals, which are more numerous in posterior half of body. (Figs. 2, 3, 4.)

9. Alimentary canal: Mouth is situated at ventro-anterior end of cephalic segment forming snout. Phalynx comes next to mouth. Oesophagus is short and slender, and passes directly to large stomach. Its connection with intestine takes place within body at level of first swimming leg. Intestine has few large constrictions in anterior portion, but proceeds as straight tube marked by mumerous ring-like constrictions and extends to boarder line of thorax and abdomen, whence it proceeds directly as rectum. Alimentary canal is composed of outer and inner layers, the former is thin and muscular, while the latter consists of a layer of rather large epithelial cells with numerous vacuoles containing green chlorophyll granules, the size of which varies very much. Regarding this granules, Leuckart, Wagner and Reinicke have already described their existence in epithelial cells of intestine of Europian species and Hartman thinks that these may be "Prosospermum", but no definite opinion is established for their real nature. Leigh Sharpe proposed that they would be a symbiotic alga and named them as *Cassatiovolvox copepodicola*, to which we wish to express our agreement, regarding various forms of granules due to different stages of this symbiotic alga. (Figs. 31, 35.)

One pair of gland exists on both sides of stomach.

10. Muscular system: In adult, well developed muscules are observed within cephalic and thoracic appendages, around vulva, anus and base of anal lamina, but none in other parts. In immature animals, muscules are seen in addition to above in thoracic and abdominal segments also.

11. Nerve system: Well developed nerves are seen to exist in immature stage, but they degenerate in adult. In adult form, nerves from supra-and suboesophageal ganglia to eyes are only present.

12. Reproductive organs: Reproductive organ in female is of the same type as in *Lernaea variabilis*, one end of ovary being connected to the wall of body cavity by means of a muscule. Oogonia-cells are present in the anterior part of ovary, whence eggs assume a chain of coin-shaped cells. Cement gland is composed of tube of swollen cells which are muscular in nature and contains a secrete cells. The secrete cell consists of bee-hive-shaped gland and is situated at the centre in the tube. Vulva is elliptical and a little prominent resembling to human ear-shell. (Figs. 14, 17.) Ovisac generally takes the shape of banana and in case of first impregnation it possesses about 20 eggs in one sac but from 270 to 300 eggs in well matured one. Egg is round or oval in shape and its size is 0.106 μ in long and 0.06 μ in short-diameter.

Development.

From well matured ovisacs nauplius hatches out. (Fig. 9.) It is elliptical, width being 0.14 mm, with length of 1.2–1.5 times of it.

First antenna is two-segmented having one spine at interior end of basal segment. Terminal joint has two plumose setae at its end and a short seta at inner margin. Second antenna has two appendages, exo-and-endopodites on basal segment. Exopodite is four-jointed and its terminal joint with a long plumose seta in centre, and short spines on both sides. One spine exists at the outer margin of other three joints. Endopodite is single-jointed, having two plumose setae at its anterior end and one spine at its inner margin. First maxilliped has four joints, its exopodite having one plumose seta at inner margin of basal joint, while single jointed endpodite having two plumose setae at its margin.

Balancer is comparatively short and is located closely to the both side of median line. (Fig. 9.)

In artificial culture conducted by the writers, nauplius has developed into metanauplius on twenty second day at 9°–13°C. Compound eyes are placed on median line close to anterior margin. (Text fig. 2, D.)

We could not observe copepod-larva by means of culture, but could find many larvae having incompletely developed horn from body of host. (Figs. 10–18.)

Lernaea elegans does not prosper in ponds of high salinity, and in examining 42 fish-rearing-ponds in consideration of relation between habitation of parasite and salinity along coast of the lake Hamana, Shizuoka prefecture, during September 1924, we have found parasites in 20 ponds whose salinity ranged between 0.0709–1.6965‰ NaCl. In 6 ponds whose salinity was found to range between 1.7889 to 7.7805‰ NaCl no parasites were observed, even though these ponds are exposed provably of infectious nature. This agrees to a large extent with result of our experiments which were carried out during last few years as regard to influence of salinity upon this parasite, and which we hope to publish before long in connection with exterminating experiments, but since the increase of salinity is the easiest method for its extermination, so we hope that this short note will prove in advance to be of some use to those who are suffering from this disease.

December, 1926.

Explanation of Plates.

Plate V.

Fig. 1. A & D. Head of *Anguilla japonica* acted upon by *Lernaea elegans*.
B. Buccal cavity of the same.
C. Head of healthy eel.

Fig. 41. Several stages of the nauplius cultured after the hatching at Nov. 5th, 1926. (Viewed from the dorsal).
A. Photographed on November 8, 1926.
B. ,, on 7 days after.
C. ,, on 10 ,, ,,
D. ,, on 18 ,, ,,

Plate VI.

Fig. 2. *Lernaea elgans* Leigh-Shape. H. horn, C. cephalon, PP. pregenital prominence, AL. anal laminae, ES. egg sac, 1–5. 1st.–5th. swimming legs, × 6.

Figs. 3–5. Pore canal. 4L. 4th. swimming leg. × 35–200.

Fig. 6. A part of ovary and oviduct. × 50.
Fig. 7. Original part of ovary. × 75.
Fig. 8. Chain of coin-shaped cells in ovary. N. nucleus, × 300.
Fig. 9. Egg in ovary. × 300.
Fig. 10. Nauplius. × 140.
Figs. 11, 12, 13, 17. Young stage. × 25.
Fig. 14. Mouth part. × 75.
Figs. 15, 16. Abdomen of young one. V. vulva, × 100.
Fig. 18. Abdomen of Fig. 17. 5L. 5th. swimming leg, V. vulva. × 100.
Fig. 19. Young stage. × 6.

Plate VII.

Fig. 20. Cephalothorax. AO. around mouth, AN. antenna, 1L. 1st. swimming leg × 140.
Fig. 21. Second swimming leg. × 300.
Fig. 22. Fouth swimming leg. × 300.
Fig. 23. Fifth swimming leg. × 300.
Fig. 24. MXP. maxilliped, B. arch-shaped bandage × 75.
Fig. 25. Gland at both side of stmach. × 140.
Fig. 26. Mandible. × 350.
Fig. 27. Second antenna. × 250.
Fig. 28. First swimming leg. × 300.
Fig. 29. Third swimming leg × 200.
Fig. 30. Muscles in swimming leg. × 200.
Fig. 31. Cross section of gland on both sides of stomach. H. horn, G. gland, S. stmach, A. symbiotic alga. × 60.
Fig. 32. Cross section of posterior part of fifth thoracic segment. I. intestin, R. rectum, CG. cement gland, PP. pregenital prominence. × 50.
Fig. 33. Longitudinal section of cephalothorax. C. cephalon, G. gland, MXP. maxilliped, 1L. first swimming leg. × 100.
Fig. 34. Cross section of middle part of fifth thoracic segment. OV. ovary, OD. oviduct, CG. cement gland. × 50.
Fig. 35. Inner layer of skin. N. nucleus. × 75.
Fig. 36. Stomach wall. A. symbiotic alga, N. nucleus. × 675.
Fig. 37. Cement gland. × 675.

Fig. 38. Longitudinal section of anterior part of body. S. stomach, I. intestin, M. muscle. × 40.

Fig. 39. Cross section of fouth thoracic segment. I. intestin, OD. oviduct. × 50.

Fig. 40. Cross section at posterior part of fouth thoracic segment. CG. cement gland, OD. oviduct, IL. inner layer, ML. middle layer, OL. outer layer, PP pergenital prominence. × 35.

Fig. 41. vid Pl. I.

ON THE NATURE OF WATER AND BOTTOM OF FISH REARING PONDS.

I. On a case of Damage done to the Cultivated Fish by Acid Soil.

(Contributions from the Toyohashi Piscicultural Experiment Station No. 2.)

By

Yoshiichi Matsui and Tokujiro Yamada.

We often hear that in the ponds in coastal districts a good many of cultivated fish die after rainfalls.

The present report is an instance of the same event that occurred in the Muro Fish Rearing Pond belonging to our Station in June 1922, and in the neighboring pond in July 1922 where the water in those ponds showed decidedly an acid reacrion.

The dead fish looked as if they were coated by a membrane due to the coagulation of viscous fluid, excepting their cornea. The gills and livers were conspicuously congested.

The analyses of the neighboring soils plainly showed the presence of acid one. We noticed a gray substance on the surface of the soil. This substance is composed of insoluble silicate and soluble salts and the later is proved to contain a large quantity of aluminium sulphate, and from the solution of the soluble salts just mentiond we obtained a beautiful hexagonal crystals. And when water contains more than 0.02% of this substance it kills eels and its reaction upon fish is the same as that of

alum; in its higher density it is slower than that of alum and vice versa. This may probably be due to the antagonism of many other salts contained in the solution than alum.

Although the damage due to the acid soil decreases year by year, yet the anhibation of the danger can be attainned in preventing rain drains from running down the banking into the pond, and also in neutralizing the soil by milk of lime on the banking.

In the case when the water of ponds turns acid, we may neutralize it by means of milk of lime also; the contents of lime should not be over 2 grs. in 100,000 c.c. of water. The toxic reaction of lime upon fishes decreases rapidly in the case wher a large quantity of organic matter is present in the fish rearing ponds.

(Abstracted from the text written in Japanese.)

November 1924.

ON THE NATURE OF WATER AND BOTTOM OF FISH REARING PONDS.

II. Analytical Table compiled up to the fiscal year 1926.

(Contributions from the Toyohashi Piscicultural Experiment Station. No. 3.)

By

Tokujiro Yamada.

The tables are the results of analysis of many samples of water and soil that were brought to our Experiment Station to ascertain their fitness for fish rearing for the construction of new ponds, and also contain the analysis made when unusual phenomena happened in the ponds. Therein also contained those tables jointly done with Mr. Y. Matsui for the Fundamental studies of water and bottomsoils of fish ponds.

The tables are put in print, though fragmentary, for the referrence to our future study. (Unit for waterquality is milligram per 1000 c.c. and for bottom-soil is gram per 100 gr.)

December 1926.

Index of Place

(1) Water.

No.

1 Nakamura fish-farm, Muroyoshida-mura, Aichi-ken.
2 Toyohashi-yogyo-shikenjyo, Kitajima near Toyohashi.
3 Maishiba-mura, Aichi-ken.
4 Toyohashi-yogyo-shikenjyo, Kitajima near Toyohashi.
5 " " " " " "
6 " " " " " "
7 Pond no. 1, Jinnoshinden-yogyo & Co. Ltd., Takashi-mura, Aichi-ken.
7 (II) Pond no A, 11, Jinnoshinden-yogyo & Co. Ltd., Takashi-mura, Aichi-ken.
8 " " 6, " " " " " " " " "
9 Fish rearing pond of Toyohashi-yogyo-shikenjyo, Muro near Toyohashi.
10 Kaneno fish-farm, Muroyoshida-mura, Aichi-ken.
11 Pond no. 13, Jinnoshinden-yogyo & Co. Ltd., Takashi-mura, Aichi-ken.
12 " " 5, Amatsushinden-yogyo & Co. Ltd., Sugiyama-mura, Aichi-ken.
13 " " 6, " " " " " " " " "
14 Fish rearing pond of Toyohashi-yogyo-shikenjyo, Mnro near Toyohashi.
15 Nakamura fish-farm, Muroyoshida-mura, Aichi-ken.
16 " " " " " " "
17 Pond no. 12, Jinnoshinden-yogyo & Co. Ltd., Takashi-mura, Aichi-ken.
18 Koiwai near Nakatsu, Oita.
19 River Sakura, Ibaragi-ken.
20 Takejiri, Gunchiku-mnra, Kumamoto-ken.
21 Branch of Kumamoto-fisheries-experiment-station, Gunchiku-mura, Kumamoto-ken.
22 Meijishinden, Gunchiku-mura, Kumamoto-ken.
23 River Rumo, Kumamoto-ken.
24 " " " "
25 Kinuura fish-farm, Takahama-mura, Aichi-ken.
26 Tokura fish-farm, Isshiki-machi, Aichi-ken.
27 " " " " " " "
28 Pond no. 18, Chūō fish-farm, Murakushi-mura, Shizuoka-ken.
29 " " 2, Hattorinakamura-yobetsu & Co. Ltd., Maisaka-machi, Shizuoka-ken.
30 " " 5, " " " " " " " " "

No.

31 Nishimaruko fish-farm, Ubumi-mura, Shizuoka-ken.

32 Chūō fish-farm, Murakushi-mura, Shizuoka-ken.

33 Araiyogyo & Co. Ltd., Arai-machi, Shizuoka-ken.

34 Funai fish-farm, Takashi-mura, Aichi-ken.

35 Toyohashi-yogyo-shikenjyo, Kitajima near Toyohashi.

36 Pond no. 7, Atsuta-fish-farm, Nagoya.

37 „ „ 11, „ „ „ „

38 Muro irrigation, Ushikawa-mura, Aichi-ken.

39 „ „ near Toyohashi-gas-company, Toyohashi.

40 Fish rearing pond of Toyohashi-yogyo-shikenjyo, Muro near Toyohashi.

41 Entrance of Muro irrigation, Hitokuwada-mura, Aichi-ken.

42 Iwata-yogyo & Co. Ltd., Fukude-mura, Shizuoka-ken.

43 Lake Kizaki, Nagano-ken.

44 „ Awoki, „ „

45 River Nogu, Nagano-ken.

46 Kizaki-yogyo-shikenjyo, Taira-mura, Nagano-ken.

47 K. Yamamoto, Toyohashi.

48 Funai fish-farm, Takashi-mura, Aichi-ken.

49 „ „ „ „ „ „ „

50 Pond no. 6, Iwata-yogyo & Co. Ltd., Obo-mura, Shizuoka-ken.

51 „ „ 10, „ „ „ „ „ „ „ „ „

52 Funai fish-farm, Takashi-mura, Aichi-ken.

53 Toyohashi-yogyo-shikenjyo, Kitajima naer Toyohashi.

54 Pond no. 14, Chuo fish-farm, Murakushi-mura, Shizuoka-ken.

55 „ „ 17, of east division, „ „ „ „

56 „ „ 6, „ west „ „ „ „ „

57 „ „ „ „ east „ „ „ „ „

58 Toyohashi-yogyo-shikenjyo, Kitajima near Toyohashi.

59 K. Okada, Toyohashi.

60 K. Okada's tap water, Toyohashi.

61 Pond no. 13, Chuo fish-farm, Murakushi-mura, Shizuoka-ken.

62 „ „ „ „ „ „ „ „ „ „

63 Lake Hamana near Chuo fish-farm, Shizuoka-ken.

64 Pond no. 12, Chuo fish-farm, Murakushi-mura, Shizuoka-ken.

No.

65 Pond no. 10, Chuo fish-farm, Murakushi-mura, Shizuoska-ken.

66 „ „ 1, „ „ „ „ „ „ „

67 Y. Yoshiwara., Takashi-mura, Aichi-ken.

68 M. Kurono., Toyohashi.

69 River Shinhori, Hirosawa-mura, Gumma-ken.

70 Chuo fish-farm, Murakushi-mura, Shizuoka-ken.

71 S. Jinno., Toyohashi.

72 Pond no. 1, Jinnoshinden-yogyo & Co. Ltd., Takashi-mura, Aichi-ken.

73 (I) Pond no. 3, Jinnoshinden-yogyo & Co. Ltd., Takashi-mura, Aichi-ken.

73 (II) „ „ B3, „ „ „ „ „ „ „ „ „

74 Pond no. 1, Jinnoshinden-yogyo & Co. Ltd., Takashi-mura, Aichi-ken.

75 „ „ 3, „ „ „ „ „ „ „ „ „

76 „ „ 1, Moroto fish-farm, Kuwana.

77 Pond no. 1, Miye-yogyo & Co. Ltd., Kuwana.

78 Branch of Miye-yogyo & Co. Ltd., Ōyamada-mura, Miye-ken.

79 Itomi fish-farm, Isojima-mura, Miye-ken.

80 Asahi-kyodokeiyei fish-farm, Isojima-mura, Miye-ken,

81 Kikuyama-yogyo & Co. Ltd., Ueno-machi, Miye-ken.

82 Ozawa fish-farm, Kamezaki-shinden, Miye-ken.

83 Matsumoto fish-farm, „ „ „ „

84 Murakushi-mura, lake Hamana, Shizuoka-ken.

85 Ubumi-mura, lake Hamana, Shizuoka-ken.

86 Centre of Ubumi bridge, lake Hamana, Shizuoka-ken.

87 Tsutsumimaruyama fish-farm, near Kiriu, Gumma-ken.

88 Suzuki fish-farm, Sachiura-mura, Shizuoka-ken.

89 Small bay Shimono, lake Biwa, Shiga-ken.

90 S. Jinno Toyohashi.

91 M. Kusogami Muroyoshida-mura, Aichi-ken.

92 Small bay Matsubara, lake Biwa, Shiga-ken.

93 Pond no. 12, Shigaken-suisan-shikenjyo, near Hikone, Shiga-ken.

94 „ „ 6, „ „ „ „ „ „ „

95 Nakamura fish-farm, Hikone-machi, Shiga-ken.

96 Fish rearing pond no. 5 of Shiga-ken-suisan-shikenjyo, around Hikone castle.

97 Small bay Shimono, Lake Biwa, Shiga-ken.

No.

98 Shimizu seishijyo, Toyohashi.
99 Drain-water of Shimizu seishijyo, Toyohashi.
100 Pond no. 5, Chūō fish-farm, Murakushi-mura, Shizuoka-ken.
101 Masuda fish-farm, Yoshida-mura, Shizuoka-ken.
102 Naito fish-farm, Muroyoshida-mura, Aichi-ken.
103 Pond no. A4, Amatsushinden-yogyo & Co. Ltd., Sugiyama-mura, Aichi-ken.
104 Masuda fish-farm, Yoshida-mura, Shizuoka-ken.
105 Fish rearing pond of Toyohashi-yogyo-shikenjyo Muro near Toyohashi.
106 „ „ „ „ „ „ „ „ „ „
107 S. Mori Muroyoshida-mnra, Aichi-ken.
108 Kirido fish-farm, Shiotsu-mura, Aichi-ken.

(2) Bottom.

No.

1 Pond no. 5, Jinnoshinden-yogyo & Co. Ltd., Muroyoshida-mura, Aichi-ken.
2 „ „ 9, „ „ „ „ „ „ „ „ „
3 „ „ 13, „ „ „ „ „ „ „ „ „
4 „ „ 2, „ „ „ „ „ „ „ „ „
5 „ „ 12, „ „ „ „ „ „ „ „ „
6 „ „ 12, „ „ „ „ „ „ „ „ „
7 „ „ 10, Tokura fish-farm, Isshiki-machi, Aichi-ken.
8 „ „ 7, „ „ „ „ „ „ „
9 Nishimaruko fish-farm, Ubumi-mura, Shizoka-ken.
10 Pond no. 18, Chūō fish-farm, Murakushi-mura, Shizuoka-ken.
11 Ōhama fish-farm, Ōhama-machi, Aichi-ken.
12 Awa fish-farm, Tsuruoka-shinden, Tokushima-ken.
13 Naito fish-farm, Muroyoshida-mura, Aichi-ken.
14 Pond no. 7, Atsuta fish-farm, Nagoya.
15 „ „ 11, „ „ „ „
16 „ „ 11, „ „ „ „
17 Pond no. 22, Jinnoshinden-yogyo & Co. Ltd., Takashi-mura, Aichi-ken.
18 „ „ 5, „ „ „ „ „ „ „ „ „
19 Funai fish-farm, Takashi-mura, Aichi-ken.
20 Funai fish-farm, Takashi-mura, Aichi-ken.
21 „ „ „ „ „ „ „

No.

22 Pond no. 6, Iwata-yogyo & Co. Ltd., Obo-mura, Shizuoka-ken.
23 „ „ 16, Chūō fish-farm, Murakushi-mura, Shizuoka-ken.
24 „ „ 17, „ „ „ „ „ „ „
25 The Pond around castle, Saga.
26 Naito fish-farm, Muroyoshida-mura, Aichi-ken.
27 Miye-yogyo & Co. Ltd., Isojima-mura, Miye-ken.
28 Itomi fish-farm, Awoshigi-mura, Miye-ken.
29 Ozawa fish-farm, Kamezaki-shinden, Miye-ken.
30 Asahi-kyodokeiyei fish-farm, Shiohama-mura, Miye-ken.
31 Kikuyamayogyo & Co. Ltd., Ueno-machi, Miye-ken.
32 Matsumoto fish-farm, Ominato-machi, Miye-ken.
33 Miyeyogyo & Co. Ltd., Oyamada-mura, Miye-ken.
34 Estimated place of a fish-farm, lake Biwa, Shiga-ken.
35 „ „ „ „ „ „ „ „ „ „
36 „ „ „ „ „ „ „ „ „ „
37 Small bay Matsubara, lake Biwa, Shiga-ken.
38 „ „ „ „ „ „ „
39 Same as Water no. 96.
40 Pond no. 6, Shigaken-suisanshikenjyo, near Hikone, Shiga-ken.
41 „ „ 12, „ „ „ „ „ „
42 Pond no. 2, Nakamura fish-farm, Hikone-machi, Shiga-ken.
43 „ „ 5, Chūmei fish-farm, Murakushi-mura, Shizuoka-ken.
44 „ „ 9, „ „ „ „ „ „ „
45 Naito fish-farm, Muroyoshida-mura, Aichi-ken.
46 Masuda fish-farm, Yoshida-mura, Shizuoka-ken.

CHEMICAL STUDIES ON THE SILKWORM-PUPA AS FISH-FOOD. I.

The difference of chemical constitution of pupa on the sort of silkworm.

(Contributions from the Toyohashi Piscicultural Experiment Station No. 4.)

By

Oshima-Shinobu.

About thirty eight million kilogrammes of dried silkworm-pupa is produced yearly as a byproduct of silk industry in Japan. Almost all of it is used as fish-food or fertilisers. Pupa is used as fish-food in two ways: wet and dried; the wet one is that which is newly obtained after realing process, and the dried is that which has been dried. All of the samples I studied here are the wet one.

We may classify them into three kinds: the annual silkworm-pupa (Spring), the bivoltini silkworm-pupa (mainly Summer), and the polivoltini silkworm-pupa (mainly Autumn); and the amount of the production of the first kind attains about fifty five percent of all, the third being about thirty, and they can easily be distinguished on external appearance. It is the main object of my study to know the difference of chemical constitutions of these three kinks. The sorts of the sample are as follows:—

No.	Sort.	Survival period after mounting.	Solubility of sericin in the coccoon.
1.	Spring. No. 1, Sort of Japan.	9 days.	A little good.
2.	Spring. No. 4, No. 7, China.	9 ,,	Good.
3.	Spring. No. 1, Japan × No. 4, China.	9 ,,	Good.
4.	Spring. No. 7, No. 9, Europe.	9 ,,	Good.
5.	Summer. No. 107, Japan × No. 101, China.	9 ,,	No Good.
6.	Autumn. (No. 9 × No. 101) China × No. 107, Japan.	8 ,,	Good.

In the next place I studied the difference of chemical constitutions of pupa in the course of survival period after mounting. The sort of it is No. 6 (The Autumn pupa).

All the samples used were obtained at the Toyokawa branch, Aichi-ken Sericultural Experiment Station and always prepared under the same treatment.

The nitrogen distribution of pupa's protein is estimated after the Van Slyke's method.

The results are shown as in Table. I–III.

The difference of six sorts of pupa accord to the differences of chemical constitutions. Among them the Spring-pupa is generally rich in dry matter especially in ether extract, but the Summer-pupa which requires longer time in realing, is poor in dry matter especially in ether-extract, while the Autumn-pupa ranks between them.

On the nitrogen distribution, there is no remarkable difference among the three kinds; but if it is compared with other edible Crustacea, we can see smaller quantity of total nitrogen as compared with the latter. This fact is due to the smaller quantity of diamino-acid.

Lastly, the longer the survival period after the mounting, the more the water is contained; at the same time, chitin and cholionin are increased more and more; on the contrary the ether-extract takes the remarkable decreasing rate.

As to the value of pupa as food, we can say the Spring-pupa is most valuable and Autumn-pupa stands in next; and as to the length of survival period, the shorter the length, the more it becomes valuable as food.

(Abstracted from the text written in Japanese.)

CHEMICAL STUDIES ON THE SILKWORM-PUPA AS FISH-FOOD. II.

The digestion of silkworm-pupa with protease of eels.

(Contributions from the Toyohashi Piscicultural Experiment Station No. 5.)

By

Oshima-Shinobu.

On other pages under a different title I have spoken about the wet and dried pupae which are used as food in fish-culture. Now I tried to examine the digestive

value in the wet and dried pupae in connection with protease of eels. The samples used are as follows :—

No. 1.	Wet pupa.	Autumn pupa.	(No. 9 × No. 101) China ; × No. 107 Japan.
No. 2.	Dried pupa. A.	Autumn pupa.	(No. 9 × No. 101) China ; × No. 107 Japan.
	B.	Summer pupa.	No. 107 Japan ; × No. 101 China.
	C.	Spring pupa.	No. 1 Japan ; × No. 101 China.

At first the materials were crushed into pieces by grinder, well rubbed out with a few water in a mortar and strained through a cloth ; then a few drops of truol were added to the mixture so as to make a liquid of a muscular suspension and a constant quantity of the liquid was taken as the material of the experiment. In the next place almost all the digestive organs of many eels were extracted in ordinary method and a constant quantity of the extract was taken. 10 c.cm. of each liquid thus prepared together with a proper quantity of water, N/10 hydrochloric acid, and N/10 sodium hydroxide were poured into a flask of 200 c.cm: in capacity as shown as in Table I. and corked and the mixture was put in a thermostat for 20 hours at the temperature of 33.5–36°C. After that time I added 10 c.cm. of tannic acid solution in them, (i.e. 100 grms. of tannic acid, 25 grms. of sodium aceticum, 35 grms. of sodium chloratum, 50 c.cm. of glacial acetic acid disolved in water of 1,000 c.cm.) in order to stop the digestive action and precipitated protein.

In the solution I estimated the quantity of the soluble nitrogen by Gunning method and amino-nitrogen by Van Slyke's micro-apparatus. On the measurement of hydrogen ion concentration, I adopted the Sorenzen's colorimetric method. The results are shown as in Table. II–IV.

In these results the protease of eels has its action on silkworm-pupa between pH 5.7 and 7.4, and seems to become more active in alkali within the limit of that hydrogen ion concentration.

In wet and dried pupae the former seem to be easily digestible but there is no marked difference for digestibility of pupae in the difference of seasons.

(Abstracted from the text written in Japanese.)

第 I 表　　TABLE I.

No.	1	2	3	4	5
水 Water.	瓦 c.cm. 20	瓦 c.cm. 19	瓦 c.cm. 18	瓦 c.cm. 19	瓦 c.cm. 18
鹽酸液 N/10 HCl Sol.	0	1	2	0	0
苛性曹達液 N/10 NaOH Sol.	0	0	0	1	2

第 II 表　　TABLE II.

消化前原料懸垂液 10 瓦中ノ窒素分布

Nitrogen distribution in 10 c.cm. of muscular suspension before the digestion.

品種 Sort.		全窒素 Total N.	蛋白質窒素 Protein N.	非蛋白質窒素 Non protein N.	あみの態窒素 Amino N.
No. I		瓦 gm. 0.03807	瓦 gm. 0.02569	瓦 gm. 0.01238	瓦 gm. 0.00263
No. II	A	0.03371	0.02439	0.00932	0.00182
	B	0.03546	0.02573	0.00973	0.00205
	C	0.04026	0.02901	0.01125	0.00248

第 III 表　　TABLE III.

消化後新生窒素ノ分布

Distribution of Nitrogen which newly produced after digestion.

品種 Sort.	No.	1	2	3	4	5
No. I	pH	6.6	6.2	5.7	6.9	7.4
	非蛋白質窒素 Non protein N.	瓦 gm. 0.00695	瓦 gm. 0.00776	瓦 gm. 0.00740	瓦 gm. 0.00663	瓦 gm. 0.00804
	あみの態窒素 Amino N.	0.00239	0.00252	0.00210	0.00211	0.00264
No. II A	pH	6.7	6.4	6.0	7.0	7.4
	非蛋白質窒素 Non protein N.	0.00590	0.00636	0.00680	0.00565	0.00675
	あみの態窒素 Amino N.	0.00192	0.00219	0.00226	0.00190	0.00215
No. II B	pH	6.7	6.4	6.0	7.0	7.4
	非蛋白質窒素 Non protein N.	0.00630	0.00638	0.00715	0.00588	0.00728
	あみの態窒素 Amino N.	0.00201	0.00213	0.00230	0.00193	0.00229
No. II C	pH	6.7	6.4	6.0	7.0	7.4
	非蛋白質窒素 Non protein N.	0.00667	0.00731	0.00818	0.00682	0.00852
	あみの態窒素 Amino N.	0.00227	0.00251	0.00280	0.00232	0.00284

第 IV 表　　TABLE IV.

No.		1	2	3	4	5
非蛋白質窒素 / 蛋白質窒素 Non Protein N. / Protein N.	No. I	% 27.2	% 30.2	% 28.8	% 25.8	% 31.3
	No. II A	24.2	26.1	27.9	23.2	27.7
	No. II B	24.5	24.8	27.8	22.9	28.3
	No. II C	23.0	25.2	28.2	23.5	29.4
あみの態窒素 / 蛋白質窒素 Amino N. / Protein N.	No. I	9.3	9.8	8.2	8.2	10.3
	No. II A	7.9	9.0	9.5	7.8	8.8
	No. II B	7.8	8.3	9.0	7.5	8.9
	No. II C	7.8	8.7	9.7	8.0	9.8

CHEMICAL STUDIES ON THE SILKWORM-PUPA AS FISH-FOOD. III.

A chemical change of stored pupae.

(Contributions from the Toyohashi Piscicultural Experiment Station No. 6.)

By

Oshima-Shinobu.

In using the pupae as fish-food in a fish-culture it is often necessary to store wet pupae for a few days and dried ones for a few month. I tried to know a change of chemical constitutions during that period.

Wet pupa. An every constant quantity of wet pupae was put in eight glass bottles and it was put under pressure a little. To four of them water was poured in the same quantity as the volume of pupae as it is usual to do so in using them as food of fishes. During the experiment temperature was carefully noted down which are shown in table. I.

Then I weighed the quantity and inspected a change of chemical constitutions of pupae during the period of the storing; by this way I tried to know the remaining constitutions and the rate of change. (Table. II.)

Dried pupa. The dried pupae were crushed into powder and put in a glass bottle, the mouth of which was tightly covered with wire guage and cloth as to prevent the entrance and damage of insects. And I studied the chemical constitutions of it after the lapse over one year. The result is as shown as in table. III.

During the period of storing, the wet pupae undergo decomposition within a few days at the beginning of the experiment and lost their value as fish-nutrition as the time passes as showe in the table II. In the dipped and non-dipped pupae the latter decompose more quickly than the former.

The dried pupae decompose slowly in the lapse of time, as it is proved in the increase of acid value of extracted oil, gradually decreasing of the quantity of ether-extract and the increase of crude ash and chitin.

(Abstracted from the text written in Japanese.)

ON THE SEASONAL CHANGE OF TEE CHMICAL CONSTITUTION OF MUSCLE OF CARPS.

(Contributions from the Toyohashi Piscicultural Experiment Station No. 7.)

By

Oshima-Shinobu.

Already we know that the spawning season and the difference of sex have a great influence on the chemical constitutions of fish muscle. I have studied the seasonal change of the chemical constitutions of muscle of carps though one year. The carps used as material have been fed in a fish pond from March to November every year. I have adopted the Van Slyke's method for the analysis of the muscle protein. The results are as shown as in Table I–III.

The dry matter is least in the quantity during the spawning season, and from that time on it increases greatly untill November when it reaches in its highest quantity; but it gradually decreases again after the fasting season. The quantity of dry matter differs between sexes, female having almost always more dry matter than male except in November. The ether-extract shows almost the same result as dry matter, but in this case it is not in November but in August that its quantity becomes largest. The seasonal change of the total-nitrogen is quite the same as the dry matter, and male contain it more than female. Every form of nitrogen changes nearly in the same manner as the total nitrogen.

In the constitution of the muscle protein, the total nitrogen is largest in quantity in August, smallest in March, and male have always more than female but only a little. But it is not always the case with the elements which constitute it. The total quantity of mono-amino-acid is the greatest in May in male, while so in March in female; and in both cases, generally saying, it is great in Spring and less in Autumn, di-amino-acid being vice versa.

Among the whole form of nitrogen in the di-amino-acid, Cystine hardly changes in its quantity through the year, female however having more tendency to change than male. Arginine and Lysine are much in male than in female, the former reaching in its highest quantity in Summer and Autumn, the latter in the spawning season, that is

第 I 表 TABLE I.

鯉 肉 成 分

Chemical Composition of Muscle of Carps in different seasons.

月別 Month.	8月 August.		11月 November.		3月 March.		5月 May.	
性別 Sex.	♂	♀	♂	♀	♂	♀	♂	♀
水分 Water.	78.20	76.03	74.22	74.26	79.21	78.46	80.57	80.13
乾物 Dry matter.	21.80	23.97	25.78	25.74	20.79	21.54	19.43	19.87
粗灰分 Crude ash.	1.21	1.03	1.28	1.39	1.15	1.12	1.01	1.18
えーてる浸出物 Ether extract.	2.30	5.74	2.07	2.41	1.50	1.95	1.31	2.66
全窒素 Total N.	2.96	2.69	3.48	3.34	2.88	2.75	2.48	2.46
蛋白質窒素 Protein N.	2.38	2.13	2.81	2.69	2.40	2.33	2.05	2.08
非蛋白質窒素 Non protein N.	0.58	0.55	0.68	0.65	0.47	0.42	0.43	0.38
可溶性窒素 Soluble N.	0.96	0.89	1.11	1.09	0.81	0.82	0.73	0.75
蛋白質窒素 Protein N.	0.38	0.34	0.44	0.44	0.34	0.40	0.30	0.37
有機鹽基窒素 Basic N.	0.25	0.22	0.29	0.29	0.18	0.17	0.18	0.18
あみの態窒素 Amino N.	0.12	0.11	0.13	0.13	0.10	0.10	0.09	0.09

第 II 表 TABLE II.

鯉 肉 蛋 白 中 ノ 窒 素 ノ 分 布

Nitrogen distribution in the Protein of Muscle of Carps in different seasons.

月別 Month.	8月 August.		11月 November.		3月 March.		5月 May.	
性別 Sex.	♂	♀	♂	♀	♂	♀	♂	♀
全窒素 Total N.	16.29	16.22	16.22	16.14	16.06	16.02	16.25	16.21
ものあみの酸窒素 Mono-amino acid N.	9.54	9.61	9.65	9.52	9.76	9.86	9.78	9.78
ぢあみの酸窒素 Di-amino acid N.	5.12	4.95	5.18	4.86	4.97	4.81	4.97	4.83
あんもにあ窒素 Ammonia N.	0.86	0.91	0.90	0.90	0.85	0.93	0.85	0.82
めらにん窒素 Melanin N.	0.77	0.76	0.75	0.84	0.49	0.57	0.65	0.78
ぢあみの酸窒素 Di-amino acid amino N.	3.08	2.91	3.07	2.86	3.02	2.86	3.03	2.88
ぢあみの酸非あみの窒素 Di-amino acid non amino N.	2.04	2.04	2.11	2.00	1.94	1.95	1.94	1.96
ものあみの酸あみの窒素 Mono-amino acid amino N.	8.65	9.05	8.57	9.00	8.92	9.25	9.02	9.25
ものあみの酸非あみの窒素 Mono-amino acid non amino N.	0.89	0.56	1.08	0.53	0.84	0.60	0.76	0.54
しすちん窒素 Cystine N.	0.05	0.06	0.05	0.06	0.06	0.06	0.05	0.06
あるぎにん窒素 Arginine N.	2.20	2.10	2.27	2.09	2.07	2.03	2.06	2.02
ひすちぢん窒素 Histidine N.	1.41	1.49	1.46	1.43	1.36	1.40	1.37	1.42
りぢん窒素 Lysine N.	1.46	1.30	1.40	1.28	1.47	1.32	1.50	1.34

第 III 表 TABLE III.

あ み の 窒 素 ト 非 あ み の 窒 素 ト ノ 比 率

Amino nitrogen divided by non amino nitrogen.

月別 Month.	8月 August.		11月 November.		3月 March.		5月 May.	
性別 Sex.	♂	♀	♂	♀	♂	♀	♂	♀
ものあみの酸 Mono-amino acid.	9.77	16.22	7.91	17.04	10.65	15.39	11.95	17.28
ぢあみの酸 Di-amino acid.	1.51	1.43	1.46	1.43	1.55	1.47	1.56	1.47

to say in May. Histidine, on the contrary, is much in female, and in Summer and Autumn it reaches its highest quantity. Melanine is very poorly in March.

(Abstracted from the text written in Japanese.)

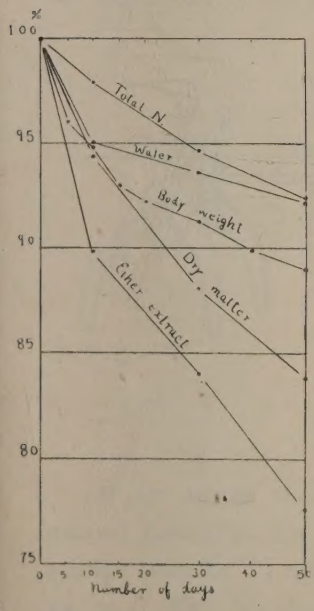
ON THE CHEMICAL CHANGE OF THE EELS IN THE COURSE OF FASTING.

(Contributions from the Toyohashi Piscicultural Experiment Station No. 8.)

By

Oshima-Shinobu.

The eels feeded in a fish pond are usually employed for cooking after a few days of fasting. So, I examined the decreasing quantity of their body weight and constituent in the course of fasting. The material I have taken had been chiefly fed on dried silkworm-pupae, fully grown up to an edible size. Three days after catching at 22nd, October, 1926, the experiment began, and continued to 11th December the same year. In the course of the fity days of the study, the temperature of water was estimated every day; because the temperature of water has a great effect on the vital phenomena of the fish. The result of the estimation of body weight which has been taken eight times is arranged in table II. Analysis has been done four times during the period, which showed the chemical constitutions of eels at each time, being given in table III. Table IV shows the decreasing quantity of each constituent of eels by calculation.



During the fasting period, eels gradually lessen their weight; and the rate of the lessening is large at the beginning of period, then decrease by degrees. This fact is

due to the consumption of their constituent, but the rate of consumption is not equal in each constituent and in each period. In water and dry matter, the latter is more consumed, consequently the more the fasting period continues, the more the difference of quantity increases. In dry matter, ether-extract consumpts much, but crude ash decreases very little; the rate of decreasing of total nitrogen is a little more than that of water. Non-protein nitrogen or soluble-nitrogen has higher rate of decreasing than protein-nitrogen or insoluble-nitrogen. Therefore the decreasing of body weight is mainly due to the decreasing of dry matter; and the decreasing of dry matter is mostly due to the decreasing of ether-extract.

(Abstracted from the text written in Japanese.)